United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service

FGIS Issuance Change

CHANGE TO

☐ DIRECTIVE ☐ MANUAL ☐ HANDBOOK

CHANGE NO:	**TO** *(No.)*	**TITLE:**	**DATE:**
8		Aflatoxin Handbook	1-5-04

PURPOSE OF CHANGE: The Aflatoxin Handbook has been revised to add test procedures for a recently approved test kit, RIDASCREEN® Fast Aflatoxin, to change references from conformance limits to "testing ranges", and to further define review inspection options.

FILING INSTRUCTIONS

Remove	Dated	Insert	Dated
Table of Contents	3-17-03	Table of Contents	1-5-04
Pages 1-3, 1-4	3-17-03	Pages 1-3, 1-4	1-5-04
Pages 1-7, 1-8	3-4-02	Pages 1-7, 1-8	1-5-04
Chapter 4	3-17-03	Chapter 4	1-5-04
Chapter 6 (Reserved)	No date	Chapter 6	1-5-04
Chapter 11	6-24-02	Chapter 11	1-5-04

Retain this issuance sheet as an aid in verifying handbook contents.

/s/ David Orr

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TABLE OF CONTENTS

1.5 APPROVED TEST METHODS

FGIS has approved test kits for use at field testing locations. The AflaCup, and Agri-Screen test kits are approved for qualitative analysis of corn. The Aflatest, Fluoroquant, Veratox-AST, Myco✓, and RIDASCREEN test kits provide quantitative analysis but can be used for qualitative results. High Performance Liquid Chromatography (HPLC) testing is reserved for quantitative testing at the Technical Services Division (TSD) only.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Approved for		Test Kit Range
	Qualitative	Quantitative	
AflaCup (International Diagnostics Inc.)	X		20 ppb
AgriScreen - (Neogen)	X		20 ppb
Veratox AST - (Neogen)	X	X	5 - 300 ppb (quantitative)
Fluoroquant - (Romer)	X	X	5 - 300 ppb (quantitative)
Aflatest – (Vicam)	X	X	5 - 300 ppb (quantitative)
Myco✓ - (Strategic Diagnostics Inc.)	X	X	5 - 80 ppb (quantitative)
RIDASCREEN Fast Aflatoxin (r-Biopharm)	X	X	5 - 50 ppb (quantitative)

NOTE: The test ranges are for performing an individual analysis with an undiluted sample extract. To obtain accurate results above the test kit range a supplemental analysis must be performed.

Listed in the table below are the test kits that are commonly used for official aflatoxin analysis. Use the table to determine the appropriate test kit(s) to use for testing the listed grain/commodity. For information concerning the testing of mixed grain, contact the Policies and Procedures Branch.

GRAIN/ COMMODITY	TEST METHOD						
	AflaCup	Aflatest	Agri-Screen	Fluoroquant	Veratox-AST	Myco✓	RIDASCREEN
Corn	X	X	X	X	X	X	X
Sorghum		X		X	X	X	X
Wheat		X		X	X		X
Soybeans		X		X	X		X
Corn Screenings		(*)			(*)		
Corn Meal		X		X	X	X	X
Corn Germ Meal		X			X		X
Corn Gluten Meal		X			X		X
Corn/Soy Blend		X		X	X	X	X
Corn Gluten Feed		X					
Flaking Corn Grits		X		(*)	(*)		
Corn Flour					(*)		
Corn Bran					(*)		
Popcorn		X		X	X	X	X
Milled Rice		X		X	X		X
Rough Rice					(*)		
Cracked Corn	(*)	(*)	(*)	(*)	(*)	(*)	X

NOTE: An X entered into a block denotes that the test kit has been evaluated and approved for the grain/commodity.

The symbol (*) entered into a block denotes that the test kit is under evaluation by TSD for the grain/commodity and is temporarily approved for official use.

Review inspection services for aflatoxin are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples.

NOTE: Do not consider any excess grain sample as a "new sample" for the basis of testing.

For submitted samples, lots that are certified on an individual carrier basis, and composite samples representing multiple carriers, a maximum of three review inspections (reinspection, appeal, Board appeal) may be performed on the original inspection service.

Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when testing is performed on a sublot basis. However, if the applicant requests a review of the entire lot, up to three review levels of service (reinspection, appeal, board appeal) may be obtained for each sublot included in the lot. Inspection results for each review level shall replace the previous inspection result.

a. Reinspection Service.

The laboratory providing original testing services also provides reinspection services. Applicants may request either qualitative or quantitative analysis unless the original test was quantitative. Then, only a quantitative analysis is available.

b. Appeal Inspection Service.

FGIS field offices provide appeal testing services for aflatoxin. Field offices not equipped to provide testing will make arrangements with another FGIS office to provide the most timely service possible. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available. If samples are sent to a field office for analysis, write the words **"AFLATOXIN APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

c. Board Appeal Inspection Services.

Board appeal inspection services are limited to the file sample and are provided by the Board of Appeals and Review (BAR) in Kansas City. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available.

The HPLC method is also available for determining aflatoxin in Board appeal samples. The applicant must specify the HPLC method as the desired determination method. Otherwise, the Board appeal inspection will be conducted using the rapid method (test kits).

When sending samples to the BAR, write the words **"AFLATOXIN BOARD APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

1.9 QUALITY ASSURANCE PROGRAM

The Technical Services Division (TSD), located at the Kansas City Technical Center, conducts an aflatoxin check sample program for all specified service points and laboratories providing testing services. TSD is responsible for preparing and distributing check samples each quarter to all official aflatoxin testing locations, analyzing check sample results, notifying field locations of any results indicating problems, and releasing a quarterly summary report to all participating laboratories. Field offices are responsible for routine supervision to assure all laboratories in their circuit provide accurate results. The TSD check sample program is designed to test the capability of the official system and to monitor the accuracy of approved testing methods. The check sample program provides limited performance information that can be used to supplement the routine supervision of official personnel performing testing services.

CHAPTER 4

CERTIFICATION

4.1 BACKGROUND

Testing performed on standardized grains (e.g., corn, wheat) is performed as an official criteria factor under the authority of the United States Grain Standards Act (USGSA), as amended. Testing performed on processed grain products (e.g., corn meal) and other commodities is provided under the authority of the Agricultural Marketing Act (AMA) of 1946, as amended.

Aflatoxin results are recorded on the pan ticket, worksheet, or loading log and in the remarks section of the certificate.

Certify aflatoxin test results on grain in accordance with the USGSA/AMA (as applicable) regulations.

Upon the request of the applicant, separate certificates may be issued for grade and for aflatoxin when both are determined on the same lot.

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

4.2 GENERAL PROCEDURES

The type of service requested and the test method used determine how aflatoxin results are recorded and certified.

a. Qualitative Testing.

(1) Record the results of a **qualitative service** on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

(2) If a **quantitative method** is used to provide qualitative service, record the test results on the work records in a quantitative measurement (e.g., 10 ppb) or a qualitative measurement (e.g., $\leq$ 20 ppb).

(3) Certify results as being equal to or less than a threshold.

b. Quantitative Testing.

Record the results on the pan ticket and the inspection log to the nearest whole ppb.

When test results indicate that aflatoxin is present at a level of less than 5 ppb, certify the results as "Aflatoxin does not exceed 5 ppb."

Certify test results that are between the lower testing range (5 ppb) and the upper testing range (e.g., 80 ppb) to the nearest whole ppb.

Test results greater than the upper testing limit of the test kit are certified as exceeding the test kit range unless a supplemental analysis is performed. For example: An aflatoxin test result of 110 ppb obtained using an aflatoxin test kit with a test range of 5 - 80 ppb would result in the following certification statement: "Aflatoxin exceeds 80 ppb."

c. Certifying Test Results of Single and Combined Lots, Unit Trains, and Shiplots.

(1) Single Lot Inspection Basis for Trucks and Railcars.

Certify each test result on a separate certificate.

(2) Combined Land Carrier Basis for Trucks and Railcars.

If an applicant requests aflatoxin testing on a composite basis (up to 5 railcars and 15 trucks) and the inspection for grade on the basis of individual carriers, factor only certificates are issued for the aflatoxin testing and separate grade certificates are issued for each carrier.

(3) Composite Sample Testing for Shiplots.

Certify the composite results using the appropriate statement.

(4) Submitted Sample Testing.

Certify the results using the appropriate statement.

(5) Unit Train and Shiplot Inspection under the CuSum Loading Plan.

(a) Sublot Size for Shiplots.

The testing frequency for shiplot grain will be the same as the sample for grade analysis unless the applicant specifically requests aflatoxin analysis on the basis of a component sample.

(b) Sublot Size for Unit Trains.

The maximum size sublot for aflatoxin testing is 5 railcars for unit trains consisting of less than 200,000 bushels, or less than 50 cars. For unit trains consisting of 200,000 bushels or more, or 50 railcars or more, the maximum sublot size is 10 railcars.

For unit trains, the sublot size for aflatoxin testing and for grade analysis may be different. For example, an applicant may request grade analysis on the basis of a sublot containing two cars and request aflatoxin analysis on the basis of five cars.

(c) Recording Test Results.

Aflatoxin test results of sublot samples taken throughout loading are recorded on the loading log. A material portion occurs if the sublot result exceeds the limit as specified in the load order.

(d) Certifying Test Results.

Certify the lot based on the mathematical/weighted average (as applicable) of the accepted sublot results.

Certify material portions separately.

(e) Material Portions.

If a material portion occurs, the applicant has the option of requesting a review inspection. Review inspection results replace previous results when determining if a material portion exists.

If a material portion designation due to aflatoxin is not removed by the review inspection process, the applicant may leave the material portion on board and receive a separate certificate; return the grain to the elevator; or discharge the material portion along with additional grain in common stowage equivalent to one half the material portion quantity.

4.3 STANDARD CERTIFICATION STATEMENTS

Use one of the applicable statements for certifying aflatoxin.

a. Qualitative Testing.

When aflatoxin results are equal to or less than a specific threshold (e.g., 20 ppb) ppb:

"Aflatoxin equal to or less than 20 ppb."

"Aflatoxin exceeds 20 ppb."

b. Quantitative Testing.

(1) When aflatoxin results are less than 5 ppb, use the following statement.

"Aflatoxin does not exceed 5 ppb."

(2) When aflatoxin test results are between 5 ppb and the upper testing range (e.g., 80 ppb) of the test kit, round to the nearest whole number in ppb.

"Aflatoxin (result rounded to the nearest whole number) ppb."

(3) When aflatoxin test results exceed the upper testing range (e.g., 300 ppb) of the test kit.

"Aflatoxin exceeds (enter upper test limit) ppb."

c. HPLC Testing.

TSD performs HPLC testing for total aflatoxins, and, upon request of the applicant for service, testing can be performed to measure individual (i.e., B1, B2, G1, G2) aflatoxin isomers in a sample. The limits of quantification for individual aflatoxin measurements are 1 ppb for aflatoxins B1 or B2, and 2 ppb for aflatoxins G1 or G2.

Use the following statement to certify total aflatoxins.

> "Aflatoxin (record actual results to the nearest whole number) ppb. Results based on High Performance Liquid Chromatography Method."

To certify a specific aflatoxin in a sample use the following statement.

> "Aflatoxin (insert B1, B2, G1, or G2 as applicable) (insert result to the nearest whole number) ppb. Results based on High Performance Liquid Chromatography Method."

To certify individual aflatoxins and the total aflatoxin concentration in a sample use the following statement.

> "Aflatoxin (insert B1, B2, G1, or G2 as applicable) (insert result to the nearest whole number) ppb, total aflatoxins (record actual results to the nearest whole number) ppb. Results based on High Performance Liquid Chromatography Method."

4.4 OPTIONAL STATEMENTS

a. Aflatoxin Not Detected.

At the request of the applicant, use the following statement when aflatoxin is not detected (0 ppb).

> "Aflatoxin not detected."

> **NOTE: If sublot results are combined and averaged and the lot average is equal to 0 ppb, but an individual sublot result exceeds 0.0 ppb, the statement may not be used.**

b. Converting to Parts per Million (ppm).

At the request of the applicant, convert and certify the ppb result to parts per million (ppm) using an approved statement. To convert ppb to ppm, divide the ppb result by 1000.

"(Actual ppb result) ppb is equivalent to (converted ppm results) ppm."

c. Converting to Milligrams (mg) per Kilogram (kg), or Micrograms (µg) per Kilogram (kg).

At the request of the applicant, convert and certify results in milligrams per kilogram (mg/kg) or micrograms per kilogram (µg/kg). Use the following equivalents to determine mg/Kg or µg /kg:

ppm = mg/kg ppb = µg /kg

"(Actual ppb result) ppb is equivalent to (converted mg/kg or µg /kg result)."

d. Multiple Results on the Same Certificate.

When certifying multiple aflatoxin results on the same certificate and the results are based on different sample types, the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: Aflatoxin equal to or less than 20 ppb."

"Composite sample result: Aflatoxin 14 ppb."

e. Negative Result Statement.

At the request of the applicant, one of the following statements may precede the applicable standard statements when test results are equal to or less than 20 ppb.

"The aflatoxin result is negative." OR "Negative aflatoxin."

f. Type of Test Statement.

At the request of the applicant, use this statement to indicate the type of aflatoxin test used.

"Results based on (indicate type of test used) method."

NOTE: These certification statements may be modified as deemed necessary.

4.5 REVIEW INSPECTION STATEMENTS

Use the appropriate statements listed below for reinspection, appeal, and Board appeal inspections.

a. Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

"This certificate supersedes Certificate No. (number) dated (date)."

b. The superseded certificate is null and void as of the date of the subsequent (reinspection/appeal/Board appeal) certificate.

"The superseded certificate has not been surrendered."

c. When a file sample is used, enter the following statement on the reinspection/appeal/Board appeal certificate:

"Results based on file sample."

d. When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/ reinspection/appeal inspection) service."

CHAPTER 6

RIDASCREEN® FAST AFLATOXIN TEST KIT

6.1 GENERAL INFORMATION

The RIDASCREEN® FAST aflatoxin test is a competitive enzyme immunoassay for the quantitative analysis of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 50 ppb.** Accurate aflatoxin measurements above 50 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

6.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the RIDASCREEN® FAST aflatoxin test is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

6.3 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 250 ml of the (70/30) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 2 minutes.

d. Filter the extract through a filtering syringe.

e. Dilute 1 ml of the filtrate with 1 ml of distilled/deionized water.

6.4 TEST PROCEDURES

a. Sample Analysis.

(1) Allow reagents, microwells, and sample extracts to reach room temperature prior to running the test.

(2) Insert a sufficient number of wells into the microwell holder for all standards and samples to be tested. (For example: to test 11 samples use 16 wells - 5 for the standards and 11 for the test samples).

Test Strip #1

Well #	1	2	3	4	5	6	7	8
Sample	C 0	C 4	C 10	C 20	C 50	S1	S2	S3

Test Strip #2

Well #	1	2	3	4	5	6	7	8
Sample	S4	S5	S6	S7	S8	S9	S10	S11

Where C 0 is the zero control, C 4 is the 4 ppb control, C 10 is the 10 ppb control, C 20 is the 20 ppb control, and C 50 is the 50 ppb control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

NOTE: Do not run more than 3 strips (19 samples) per set of control standards.

(3) Using a new pipette tip for each standard and sample, pipet 50 µl of standards and prepared sample to separate wells.

(4) Add 50 µl of enzyme conjugate (red capped bottle) into each well.

(5) Add 50 µl of anti-aflatoxin antibody (black capped bottle) into each well.

(6) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(7) Incubate for 5 minutes (± 0.5 minutes) at room temperature.

(8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.

(9) Using a wash bottle, fill each well with distilled or deionized water. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).

(10) Add 100 µl of substrate/chromagen (white dropper bottle) to each well.

(11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(12) Incubate for 5 minutes (± 0.5 minutes) at room temperature (64 – 86º F). Cover the wells with a paper towel to protect them from light sources.

(13) Add 100 µl of stop solution (yellow or orange dropper bottle) to each well.

(14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.

(15) Measure absorbance at 450 nm using the Biotek EL 301, or Awareness Technology Stat-Fax Model 303 PLUS microwell readers.

(Results must be read within 10 minutes)

b. Reading Results with the Microwell Reader.

(1) Biotek EL 301 Microwell Reader.

(a) Make sure that the microwell reader is on and allowed to warm-up for a minimum of 15 minutes before using.

(b) Remove sample carriage and hit "Enter."

(c) Insert W2 filter and hit "Enter."

(d) Insert W1 filter (450 nm) and hit "Enter."

(e) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.

(f) Load microwells into sample carriage so that the first control labeled 0 is in position A1.

(g) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.

(h) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.

(i) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.

(j) Repeat step (i) until absorbance values have been obtained for all controls and samples. Record the values.

(k) Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

(2) Stat-Fax Model 303 PLUS Microwell Reader

(a) To begin from the "Ready" prompt, press Menu, key in the test number, and then press Enter.

(b) The screen will read, "Set carrier to A, press enter." Place the wells all the way to the right in the carrier. Push the carrier all the way to the left to line up the notch with the wells, then press enter. The carrier will advance into the reader, and it should start to print.

(c) When the reader is finished reading the strip, the screen will read, "Plot Curve Y/N?"

Press "Yes" (1/A) to print the graph.

Press "No" (0) to skip this feature.

(d) The screen will read, "Accept Curve Y/N ?"

Press "Yes" (1/A) to accept the curve and proceed to read another strip. When finished reading the second strip, press "Clear" twice and the results strip will print, "Test Ended."

Press "No" (0) to end the test.

6.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 50 ppb are reported as >50 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

6.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 50 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 50 ppb, the sample extract must be diluted so that a value between 5 and 50 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 70 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 34 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 68 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

True Aflatoxin Value = (10 ÷ 5) x 34 ppb
= 2 x 34 ppb = 68 ppb

6.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

6.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the syringe into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the remaining ground portion must be decontaminated, using bleach, prior to disposal. Discard the filter syringe and remaining ground portion into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

6.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

(1) 1 microtiter plate.

(2) 48 antibody coated microwells.

(3) 5 aflatoxin standard solutions of 1.3 ml each; 0, 4, 10, 20, and 50 ppb aflatoxins.

(4) 1 red-capped bottle of 3 ml peroxidase conjugated aflatoxin solution.

(5) 1 black-capped bottle of 3 ml anti-aflatoxin antibody.

(6) Microwell holder.

(7) 1 white dropper bottle of 6 ml Substrate/Chromagen.

(8) 1 yellow or orange dropper bottle of Stop reagent.

b. Materials Required but not Provided.

(1) Methanol - ACS grade or better.

(2) Deionized or Distilled Water.

(3) 250 ml graduated cylinder.

(4) 125 ml container.

(5) Filtering syringe (JM1000).

(6) Sample collection tubes.

(7) Waring high-speed blender with a one liter jar, or equivalent.

(8) Sample grinder.

(9) Balance.

(10) Biotek EL 301 or an Awareness Technology Inc. Stat-Fax Model 303 Plus Microwell reader equipped with a 450-nm filter.

(11) Eppendorf Repipettor, or equivalent, and 2.5 ml syringes.

(12) 50 µl and 1000 µl Pipettor and pipette tips.

(13) Paper towels, Kaydry paper or equivalent absorbent material.

(14) Waste receptacle.

(15) Timer: 3 channel minimum.

(16) Waterproof marker, Sharpie or equivalent.

(17) Wash bottle.

(18) Deionized or distilled water.

6.10 STORAGE CONDITIONS

a. Storage Conditions.

(1) The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 35° F and 46° F. **(DO NOT FREEZE)**

(2) Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.

(3) The substrate/chromogen solution is light sensitive, therefore, avoid exposure to direct light.

b. Indication of Instability or Deterioration of Reagents.

(1) Any bluish coloration of the red stained substrate/chromogen solution is indicative for deterioration and the reagent should be discarded.

(2) A value of less than 0.6 absorbance units for the zero standard may indicate deterioration of reagents.

CHAPTER 11

MYCO✓ TEST KIT

11.1 GENERAL INFORMATION

The Myco✓ test is a competitive enzyme-linked immunosorbent assay that provides quantitative measurement for the presence of aflatoxin in select grains and commodities. **The test kit is limited to providing aflatoxin measurements between 5 – 80 ppb.** Accurate aflatoxin measurements above 80 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

11.2 PREPARATION OF SOLUTIONS

a. Extraction Solution.

The extraction solvent used in the Myco✓ test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

(1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

(2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Wash Solution.

(1) Transfer the contents of the Wash Concentrate vial to a 500-ml plastic squeeze bottle and add 475 ml of distilled or deionized water.

(2) Swirl to mix.

11.3 EXTRACTION PROCEDURES

a. Place a sheet of filter paper (Whatman #1 folded or equivalent) into a funnel mounted over a clean collection container.

b. Label the collection container with the sample identification.

c. Transfer 50 grams of ground sample into an extraction mixing jar.

d. Add 250 ml of the (70/30) methanol/water extraction solvent.

e. Cover the extraction jar and blend on high speed for 2 minutes.

f. Allow the extract to stand for 2-3 minutes to allow the slurry to settle.

g. Filter a minimum of 15 ml of the extract into the collection container.

11.4 TEST PROCEDURES

a. Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test.

b. Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

NOTE: The maximum number of test samples that can be run at one time is 19. Using a strip of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.

c. Using a pipette, dispense 150 µl of Enzyme Conjugate into each red mixing well.

d. Dispense 50 µl of each calibrator and sample into the appropriate red mixing wells using an adjustable or fixed 50 µl pipette.

NOTE: Use a clean pipette tip for each addition.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
mixing wells	O	O	O	O	O	O	O	O	O	O	O	O
	C0	C10	C20	C40	C80	S1	S2	S3	S4	S5	S6	S7

Where C0 is the zero control, C10 is the 10 ppb control, C20 is the 20 ppb control, C40 is the 40 ppb control, and C80 is the 80 ppb control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

e. Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.

f. Using a multi-channel pipette, transfer 100 µl of each reaction mixture directly into the corresponding clear test wells. Discard the mixing wells into an appropriate waste container.

g. Let the reaction mixture incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

h. At the end of the 5-minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500-ml squeeze bottle containing wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash for a **total of four washes**.

i. After the last wash, invert the wells and tap on absorbent paper to remove residual wash solution. Wipe excess liquid from the bottom of the wells.

j. Pour substrate solution into a clean reagent reservoir.

k. Dispense 100 µl of substrate solution into each test well using a multi-channel pipette.

l. Let the substrate solution incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.

m. Pour stop solution into a clean reagent reservoir.

n. Dispense 100 µl of stop solution into each test well using a multi-channel pipette.

o. Read and record the optical density of the wells at 650 nm using a Hyperion MicroReader™ 3 well reader. Make sure that the well bottoms are clean and dry before placing in the reader. Read the test results within 20 minutes of test completion. Use the data reduction software provided by SDI to quantify results.

11.5 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 80 ppb are reported as >80 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

11.6 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 80 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 80 ppb, the sample extract must be diluted so that a value between 5 and 80 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 54 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 108 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

$$\text{True Aflatoxin Value} = (10 \div 5) \times 54 \text{ ppb}$$
$$= 2 \times 54 \text{ ppb} = 108 \text{ ppb}$$

11.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

11.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

11.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

(1) 48 antibody-coated microtiter wells.

(2) 48 red-marked mixing wells.

(3) 5 vials each containing 2 ml of 0, 10, 20, 40, and 80 ppb of aflatoxin calibrators.

(4) 1 vial containing 8 ml of aflatoxin-HRP enzyme conjugate.

(5) 1 vial containing 8 ml of substrate.

(6) 1 vial containing 8 ml of stop solution.

(7) 1 vial containing 25ml of 20X wash concentrate.

(8) 4 multi-channel pipette reservoirs.

b. Materials Required but not Provided.

(1) Methanol - ACS grade or better.

(2) Deionized or distilled water.

(3) 100 ml graduated cylinder.

(4) Whatman #1 filter paper or equivalent.

(5) Glassware with 125 ml capacity for sample extraction.

(6) Filter funnel.

(7) 50 µl pipette with disposable tips.

(8) 50 -200 µl multi-channel pipette.

(9) 500 ml plastic squeeze bottle.

(10) Blender with mixing jars.

(11) Balance.

(12) Sample grinder.

(13) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.

(14) Timer.

(15) Waterproof marker.

(16) Microwell holder.

11.10 STORAGE CONDITIONS

a. Store test kits between 36°- 46° F when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits.

b. Do not use reagents from other SDI aflatoxin kits with different lot numbers.

c. Bring kits up to room temperature 64°- 86° F prior to use.

d. Do not use kit components beyond their expiration date.